

CLAIMS

1. A process for the production of a polyunsaturated fatty acid (PUFA), the process comprising culturing a micro-organism in a culture medium inside a fermentation vessel, whereby at a stage which precedes the end of fermentation;
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- a) the carbon source is consumed by the micro-organisms at a rate greater than it is added to the medium;
  - b) the carbon source is added at a rate of  $\leq 0.30\text{M carbon/kg medium per}$   
10 hour;
  - c) the carbon source is rate limiting on the growth of the micro-organisms, or is restricted such that the micro-organisms metabolise fat(s) and/or lipid(s);
  - d) the rate of addition of the carbon source is reduced or is below the rate of  
15 consumption of the carbon source by the micro-organisms; or
  - e) the carbon source has been all used, or has a concentration in the medium of about zero, at or before the end of fermentation;
  - f) the carbon source addition is stopped but fermentation is allowed to continue; and/or
  - 20 g) the micro-organisms are subjected to conditions whereby they metabolise, or consume, one or more fat(s) or lipids(s) in preference to arachidonic acid (ARA).
2. A process according to claim 1, wherein:
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- h) the concentration of the carbon source in that stage is on average  $\leq 10\text{g/kg}$  and/or  $\leq 0.17\text{ M carbon/kg medium}$ ;
  - i) the carbon source is glucose; and/or
  - j) the PUFA is present in a microbial oil.
- 30 3. A process according to claim 1 or 2, wherein:
- k) the second stage starts at from 15 to 2 hours before the end of the fermentation or less than 10 days after the beginning of the fermentation.

4. A process according to any one preceding claim, wherein:
- l) the (entire) fermentation is carried out at a temperature  $\geq 22^{\circ}\text{C}$  or and/or  $\leq 30^{\circ}\text{C}$ ;
  - 5 m) it is conducted in the absence of an additive oil; and/or
  - n) the fermentation lasts no longer than 9 days.
5. A process according to any preceding claim wherein:
- n) the PUFA comprises arachidonic acid (ARA); and/or
  - 10 o) the vessel has a capacity of at least 10 litres.
6. A process according to any preceding wherein the micro-organism is *Mortierella*, optionally *Mortierella alpina*, and/or is non-genetically modified.
- 15 7. A microbial oil which comprises at least 50% arachidonic acid (ARA) and:
- a) has at least a 90% triglyceride content;
  - b) has a peroxide value (POV) of no more than 2.5;
  - c) has an anisidine value (AnV) of no more than 1.0;
  - d) is hexane extracted; and/or
  - 20 e) has a phospholipid content below 5%.
8. An oil according to claim 7 which comprises:
- f) less than 5% of  $\text{C}_{20}$  and/or  $\text{C}_{24}$  polyunsaturated fatty acids (PUFAs); and/or
  - 25 g) less than 5% of  $\text{C}_{22+}$  PUFAs.
9. An oil according to claim 7 or 8 wherein:
- h) the free fatty acid content is  $\leq 0.4\%$ ;
  - i) the triglyceride content is at least 95% or 98%; and/or
  - 30 j) the oil is preparable by a process according to any one of claims 1 to 6.

10. A composition comprising a microbial oil obtainable by a process according to any one of claims 1 to 6 and/or a microbial oil according to any one of claims 7 to 9.

5 11. A composition according to claim 10 which is a foodstuff (such as an infant formula), food, feed, or feed supplement, pharmaceutical, veterinary or cosmetic composition.